

groups II (55%), III (65%). The changes could be related to primary angioplasty, systematic since 2000.

Conclusions — Induction of ventricular flutter or fibrillation is actually rarer than in years 1985/2000. The decrease corresponded with the development of primary angioplasty and revascularization. The induction of monomorphic VT < 270 b/min has not changed although a lower LVEF in patients studied since 2000.

G009

REGULATION OF THE CARDIAC SODIUM CHANNEL NAV1.5 BY A MEMBER OF MAGUK PROTEINS: SAP97

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Nav1.5 is the voltage-gated sodium channel that initiates the cardiac action potential. A precise regulation and localisation of Nav1.5 channels in cardiomyocytes is thus necessary for correct heart function. The three last amino-acids of Nav1.5 (SIV) constitute a PDZ-domain binding motif known to interact with the syntrophin-dystrophin complex and PDZ domains found in proteins of the MAGUK family. Among their multiple roles, MAGUK proteins can cluster proteins and localize them at the plasma membrane.

We investigated the interaction between SAP97, one cardiac MAGUK protein, and Nav1.5. We postulated that this interaction may be implicated in correct localisation, anchoring, turn-over and/or regulation of Nav1.5 biophysical properties.

Pull-down experiments performed with Nav1.5 C-terminus fusion proteins and human or mouse heart protein extracts revealed that the association between SAP97 and Nav1.5 depends on the PDZ-domain binding motif of Nav1.5. This interaction was specific for SAP97 and Nav1.5 as no pull-down could be detected with PSD95 or ZO-1, two MAGUK proteins also expressed in human heart. The functional consequences of this interaction were studied via patch-clamp experiments. Silencing of SAP97 reduced the whole-cell sodium current measured in HEK293 cells stably expressing Nav1.5 channels without decreasing the total protein amount. In control or silenced HEK293 cells, sodium current produced by Nav1.5 δ SIV channels was reduced compared to WT. Immunostainings on frozen mouse heart slices demonstrated the colocalisation of Nav1.5 and dystrophin specifically at lateral membranes, but not at the intercalated discs. The possible colocalisation of Nav1.5 and SAP97 at the level of intercalated disks is currently investigated. This would support the hypothesis of the presence of two pools of Nav1.5 channels: one targeted at lateral membranes by the syntrophin-dystrophin complex, and another one targeted at intercalated discs by SAP97.

These findings strongly support the existence of an interaction between Nav1.5 and SAP97 in cardiac tissue. This interaction also depends on the presence of Nav1.5 PDZ-domain binding motif and may play a role in determining the channel density at the plasma membrane. Additional biochemistry, cytochemistry and biophysical experiments will allow us to further address this question.

G010

REGULATION OF VOLTAGE-DEPENDENT CALCIUM CHANNELS BY NEDD4-1

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Calcium entry into excitable cells can be regulated by controlling both the activity of calcium channels and the amount of channels available at the plasma membrane. Little is known about their internalisation and degradation. One post-translational modification shown to be involved in membrane protein internalisation and subsequent degradation is the attachment of ubiquitin moieties by ubiquitin-ligases. Previously, it has been shown that cardiac ion channels such as Nav1.5 and KCNQ1 are down-regulated by ubiquitin-ligases of the Nedd4 family. These regulations involve the interaction between the PY motif of the target channels and the WW motif of the ubiquitin-ligases. Despite the absence of such PY motif in the cardiac voltage-gated calcium channel Cav1.2, we investigated whether this channel could be regulated in the same manner than other cardiac channels as previously described.

We co-expressed, in HEK293 cells, L-type calcium channels and its two regulatory subunits Cavbeta and Cavalpha2delta1 together with ubiquitin-ligases, and examined by voltage-clamp whole-cell recordings the calcium current. We next determined by western blot and surface biotinylation assays the availability of the different subunits of calcium channels in total HEK293 lysates, and at the cell surface. Levels of ubiquitylation of the different subunits were assessed by pull-down GST-S5A and immunoprecipitation of Cav1.2 and its subunits.

We found that co-expressing the ubiquitin-ligase Nedd4-1 significantly reduced Cav currents, and decreased Cavalpha and its subunit protein levels. This effect was Nedd4-1 specific since none of the other members of the Nedd4 family we tested produced a similar effect. We also found that the effect of Nedd4-1 was dependent on the co-expression of the Cavbeta subunit. No Nedd4-1-dependent increase in ubiquitylation of the Cavalpha protein was found; and unexpectedly, the two regulating subunits Cavbeta and Cavalpha2delta1 were detected to be deubiquitylated upon Nedd4-1 co-expression.

Our data suggest that Nedd4-1 regulates the expression of Cav channels and its subunits by Nedd4-1 via an indirect mechanism constituting a new regulatory pathway to be determined. Further experiments will focus on the role of adrenergic receptors known to be ubiquitylated by Nedd4 and to bind to Cav1.2.

G011

INCIDENCE AND PROGNOSIS OF VENTRICULAR TACHYCARDIA IN APPARENTLY NORMAL SUBJECTS

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The prognosis of ventricular tachycardia (VT) is related to the presence of heart disease (HD). VT in patients without HD is considered as benign. However some sudden deaths were reported. The development of new methods as cardiac MRI has permitted to detect HD in some patients. The purpose of the study was to evaluate the incidence and prognosis of patients recruited since 30 years for VT and without apparent HD with conventional methods.